

Quantification of total Phenolic and Flavonoid Contents, and GC-MS Profile of Extracts of Piper Guineense, Dennettia Tripetala and Ocimum Gratissimum used in the Control of Cowpea Bruchid Callosobruchus Maculatus in Storage Grains

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Abstract - Certain plants have been shown to possess insecticidal properties and the phytochemicals obtained from these plants have the advantage of serving as new leads for the design of target-specific molecules that will exhibit new modes of action against grain pest. Seeds of *Piper guineense* Schum and Thonn (PG), fruits of *Dennettia tripetala* Baker (DT) and leaves of *Ocimum gratissimum* Linnaeus (OG) were dried to constant weight and ground into powder. Each of the powders was soaked separately in n-hexane and methanol and the extracts obtained were concentrated by distillation and evaporated to dryness using a rotary evaporator. Phytochemical screening of the extracts was carried out and some of the identified phytochemicals quantified using a spectrophotometer. The phytochemical profile of the extracts was determined using GC-MS. Flavonoids were identified in the methanol extracts of PG seeds, fruits of DT and leaves of OG; Steroids in methanol extract of PG and n-hexane extracts of PG and DT; Tannins and saponins in methanol extract of OG and terpenoids in methanol extract of PG and n-hexane extracts of OG and DT. Alkaloids and glycosides were absent in all the extracts. The total phenolic content of the methanol extracts of OG, PG and DT were 21.48 ± 0.15 , 67.48 ± 0.12 , 13.09 ± 0.11 mg Gallic acid equivalent /g extract while the Total flavonoid content were 0.15 ± 0.01 , 4.23 ± 0.02 , 1.69 ± 0.01 mg quercetin equivalent/g extract respectively. A total of 35 and 23 compounds were separated while 16 and 12 compounds were identified in the methanol extracts of OG and DT. A total of 55 compounds were separated and 20 identified in the n-hexane extract of PG. The identified compounds with the highest quantity in its extract were n-hexadecanoic acid (10.24%), N, N'-dimethyl-2-butene-1,4-diamine (15.99 %) and 6-(1-adamantylamino)-

Furazano[3,4-b]pyrazin-5-ol (11.51%) in PG, DT and OG respectively. The identified compounds are suspected to be responsible for the insecticidal activity of each of the plant parts which controlled *Callosobruchus maculatus* in storage grains.

Keywords - *Piper guineense*, *Dennettia tripetala*, *Ocimum gratissimum*, n-Hexane, Methanol, Extracts, *Callosobruchus maculatus*, Phytochemicals, GC-MS

I. INTRODUCTION

For many years, various researchers have reported the use of derivatives from plants in form of oils, essential oils, powders and extracts either as medicinal directly for man's consumption, to cure a disease or ailment or as part of the constituents of drugs manufactured by pharmaceutical industries (Calixto, 2019). All over the world, herbs have been accepted as important aspect of medicine because these plants have the ability to produce chemicals which has helped man in the proper functioning of his body (Li and Vederas, 2009). Most developing countries use herbs for treating diseases as such World Health Organization (WHO) as given her concert to its consumption (Newman and Cragg, 2016). These herbs could also be very useful in the protection of food crops from destruction by pests. *Callosobruchus maculatus* a cosmopolitan beetle which causes total destruction of cowpea in storage between 3 and 9 months have continually challenged the capacity of man to put it under control over the years (Dawodu and Ofuya, 2000; Ogunkoya and Dawodu, 2014).

Interestingly, man has stood up to the challenge by using synthetic insecticides (Biolo and Bertola, 2015).



Unfortunately, chemical control method had caused more damage to man than good when the protected food is consumed due to the presence of chemical residues in food in the wake of protection (Babarinde *et al.*, 2018). These chemicals work the same way in man when ingested as organophosphates and carbamates insecticides as they cause cholinergic syndrome symptoms in man (Sharifzadeh *et al.*, 2017). Other severe symptoms include headaches, blurred vision, slurred speech, convulsion, blockage of the respiratory path, slow delivery of neuropathic messages leading to Parkinson and Alzheimer diseases and coma in some very serious situations (Abdollahzadeh *et al.*, 2015). Other reproductive effect may be mimicry, blockage of hormones or triggering of inappropriate hormonal activity which may cause sterility, low sperm count, cancer of the reproductive organs, or impotence (Bjorling-Poulsen *et al.*, 2008; PAN, 2012).

These and many more effects on man are the consequences of controlling *C. maculatus* with synthetic insecticides over the years (Alao and Adebayo, 2011). As such, there is the need to develop other methods for the protection of stored grains from grain destructive insects. The use of herbals for protection of stored grains against pests is increasing among large scale farmers and it is important to know the phytochemical profile and quantity of these phytochemicals present in these herbs. Therefore, the aim of this research is to determine the phytochemical profile and quantity of these phytochemicals present in *Piper guineense*, *Dennettia tripetala* and *Ocimum gratissimum* which are herbals used for the control of stored grain pests.

II. METHODOLOGY

A. Sample collection and preparation

The plant samples used for this research were *Piper guineense* seeds (PG), *Dennettia tripetala* fruits (DT) and *Ocimum gratissimum* leaves (OG). These samples were collected from Forest Research Institute of Nigeria (FRIN) Ibadan. Authentication of each plant material was done at the herbarium of the Department of Pure and Applied Biology, Ladoké Akintola University, Ogbomoso, Nigeria. The plants samples were air dried under laboratory condition for 3 weeks. The dried samples were then ground into powder. The powder samples were stored in clean containers with tightly fitted lids until further use.

B. Extraction

200g of each of the ground plants samples were successively extracted with n-hexane and methanol respectively using a Soxhlet extractor. Each of the extracts were concentrated by distillation using a rotary evaporator and finally by evaporation to dryness. The dried extracts were weighed and stored in a cool dry container.

C. Phytochemical analysis

a) Phytochemical screening

The plants samples were subjected to phytochemical screening using the method described by Harborne, 1999. The samples were screened for presence

of certain classes of phytochemicals such as flavonoids, steroids, tannins, terpenoids, alkaloids, saponins and glycosides.

b) GC-MS analysis

An Agilent (USA) gas chromatograph (7890A) hyphenated to a mass spectrophotometer (5975C) that is equipped with an auto injector coupled with a triple axis detector was used. The capillary column specifications and other GC-MS conditions are as follows: Capillary: column length - 30m, internal diameter - 0.2µm, thickness - 250µm, column temperature started at 35°C for 5mins and changed to 150°C at the rate of 4°C/min, later raised to 250°C at the rate of 20°C/min and held for 5mins; ion source and interface temperatures - 250, 300°C; pressure - 16.2psia; out time - 1.8 min; 1µl injector in split mode with split ratio - 1:5; injection temperature - 300°C; elution time - 47.5mins.

D. Determination of total phenolic content

5ml of methanol was added to 5 mg of each sample and mixed using a vortex mixer. 0.5ml of this solution was introduced into a test tube containing 3.5ml distilled water and 0.25ml Folin-Ciocalteu reagent, mixed properly and incubated for 10mins at room temperature. 0.75ml of 20% NaCO₃ was added to the mixture and incubated for 2h. Later the absorbance was measured at 765 nm against a reagent blank. The total phenolic content was obtained from a calibration curve of Gallic acid and expressed as mg Gallic acid equivalent/g weight of dry extract.

E. Determination of total flavonoid content

10 mg of each of the extracts were dissolved in 10 ml of methanol and mixed properly using a vortex mixer. 0.5 ml of the dissolve extract was added to a mixture of 1.5 ml methanol, 0.1 ml of 10% AlCl₃, 0.1 ml potassium acetate and 2.8 ml distill water and incubated for 30mins at room temperature. The absorbance was measured against a reagent blank at 415nm. A calibration curve of quercetin was obtained and the total flavonoid content calculated from this curve and expressed as mg quercetin equivalent/g weight of dry extract.

III. RESULTS

The phytochemical screening of the methanolic and n-hexane extracts of *O. gratissimum*, *P. guineense* and *D. tripetala* showed presence of flavonoids, steroids, tannins, saponins and terpenoids while alkaloids and glycosides are absent as presented in Table 1.

Table 1: Phytochemicals from *O. gratissimum* leaves, *P. guineense* seeds and *D. tripetala* fruits extracts

Extracts	Flav	Ste	Tan	Sap	Alk	Gly	Ter
OG Met	+	-	+++	++	-	-	-
OG Hex	-	-	-	-	-	-	+
PG Met	++	+++	-	-	+	-	++
PG Hex	-	++	-	-	-	-	+++
DT Met	+	-	-	-	-	-	-
DT Hex	-	+	-	-	-	-	+

Keys: OG– *Ocimum gratissimum*, PG– *Piper guineense*, DT– *Denettia tripetala*, Met – methanol, Hex – n – hexane, Flav – flavonoid, Ste-steroid, Tan – tannin, Sap–saponin, Alk–alkaloid, Gly–Glycoside, Ter– Terpenoid, + – present, ++ – moderately present, +++ – highly present, - - absent.

Total phenolic and flavonoid content and antioxidant activity of methanolic extract

The total phenolic and flavonoid contents of methanolic extracts of *O. gratissimum*, *P. guineense* and *D. tripetala* are shown in Table 2. That of *P. guineense* gave the highest total phenolic and flavonoid contents while *D. tripetala* presented the lowest total phenolic and *O. gratissimum* the lowest total flavonoids.

Table 2: Total phenolic and flavonoid contents of the methanolic extracts *O. gratissimum* leaves, *P. guineense* seeds and *D. tripetala* fruits

Extracts	TPC (mg) Gallic acid equivalent /g extract	TFC (mg) quercetin equivalent/g extract
OG	21.48 ± 0.15	0.15 ± 0.01
PG	67.48 ± 0.12	4.23 ± 0.02
DT	13.09 ± 0.11	1.69 ± 0.01

TPC – Total Phenolic content, TFC – Total flavonoid content, OG – *Ocimum gratissimum* methanol extract, PG – *Piper guineense* methanol extract, DT – *Denettia tripetala* methanol extract

The GC-MS analysis of extracts of *O. gratissimum*, *P. guineense* and *D. tripetala* are presented in Tables 3-6. The identified compounds are carbonyls, nitrogenous, amino acids, fatty acids, alkaloids and terpenoids.

Table 3: Identified compounds from GC-MS analysis of methanolic extract of *Piper guineense* seeds

Serial No	Retention time (min)	Compound Name	Peak height	Percentage composition	Molecular formula
1.	7.814	2-furanmethanol	16929178	2.35	C ₅ H ₆ O ₂
2.	8.546	4-cyclopentene-1,3-dione	20337198	2.33	C ₅ H ₄ O ₂
3.	8.671	4-cyclopentene-1,3-dione	19431459	1.41	C ₅ H ₄ O ₂
4.	9.991	2(5H)-furanone	17137706	1.85	C ₄ H ₂ O ₂
5.	11.559	5-methyl-2-furan carboxaldehyde,	9505872	0.64	C ₆ H ₆ O ₂
6.	11.693	4-methyl-2-furan carboxaldehyde,	14847278	0.80	C ₆ H ₆ O ₂
7.	13.988	2-(1,1-dimethylethyl) Aziridine, 3-methyl-trans	19199357	1.87	C ₇ H ₁₅ N
8.	16.528	Glycylsarcosine	9704483	0.76	C ₅ H ₁₀ N ₂ O ₃
9.	16.866	1-(2-methyl-4-nitroimidazol-5-ylthio)-3-(1-piperidyl)propan-2-ol	10774168	0.58	C ₁₂ H ₁₉ N ₄ SO ₃
10.	17.110	3,7-dimethyl-1,6-octadien-3-ol,	12872588	0.94	C ₁₀ H ₁₈ O
11.	18.580	Piperidine carboxaldehyde	12245276	0.84	C ₆ H ₁₁ NO
12.	24.234	Formic acid, (2-methoxyphenyl) methyl ester	24752074	1.73	C ₉ H ₁₀ O ₂
13.	24.810	2-methoxy-4-vinylphenol	53876704	3.84	C ₉ H ₁₀ O ₂
14.	25.348	Piperonal	22678132	1.44	C ₈ H ₆ O ₃
15.	28.050	Caryophyllene	27161071	1.56	C ₁₅ H ₂₄

16.	29.289	Cis- β -farnesene	1831639	1.16	C ₁₅ H ₂₄
17.	30.089	1-(1,5-dimethyl-4-hexenyl)-4-methylbenzene	15418606	0.75	C ₁₅ H ₂₂
18.	30.415	1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-[2R-(2a,4 α ,8 β)]naphthalene	9310090	0.66	C ₁₅ H ₂₄
19.	30.890	β -Bisabolene	67992850	4.01	C ₁₅ H ₂₄
20.	31.347	3-(1,5-Dimethyl-4-hexenyl)-6-methylene-[S-(R*,S*)]Cyclohexene	76920454	5.10	C ₁₅ H ₂₄
21.	32.548	3,7,11-Trimethyl-(E)-1,6,10-Dodecatrien-3-ol	17865499	0.88	C ₁₅ H ₂₆ O
22.	33.061	Caryophyllene oxide	9740647	0.66	C ₁₅ H ₂₄ O
23.	34.518	Apiol	17027756	1.09	C ₁₂ H ₁₄ O ₄
24.	38.872	n-Hexadecanoic acid	152557396	10.24	C ₁₆ H ₃₂ O ₂
25.	39.992	Vitamin E	45822508	1.02	C ₂₉ H ₅₀ O ₂
26.	43.407	3 β -Stigmast-5-en-3-ol	54550560	9.27	C ₂₉ H ₅₀ O

Table 4: Identified compounds from GC-MS analysis of n-hexane extract of *P. guineense* seeds

Serial No	Retention time (min)	Compound Name	Peak height	Percentage composition	Molecular formula
1.	24.353	2-iodoethylbenzene	76513413	1.99	C ₈ H ₉ I
2.	25.848	α -Copaene	38328734	0.79	C ₁₅ H ₂₄
3.	28.569	α -Muurolene	166274600	2.33	C ₁₅ H ₂₄
4.	28.676	γ -Elemene	114279664	2.86	C ₁₅ H ₂₄
5.	28.957	γ -Muurolene	87196336	1.87	C ₁₅ H ₂₄
6.	30.364	1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)[1S-(1 α ,7 α ,8 α)]naphthalene	255879356	7.18	C ₁₅ H ₂₄
7.	32.191	2,4a,5,6,7,8,8a-octahydro-3,5,5-trimethyl-9-methylene (4aS-cis)1H-benzocycloheptene	169805263	2.12	C ₁₅ H ₂₄
8.	32.404	4-ethenyl- α , α -4-trimethyl-3-(1-methylethenyl)-[1R-(1 α ,3 α ,4 β)]Cyclohexane	96617632	1.15	C ₁₅ H ₂₆ O
9.	32.466	Cis-sesquibabinene hydrate	62738788	0.88	C ₁₅ H ₂₆ O
10.	33.892	Guaiol	104812320	2.09	C ₁₅ H ₂₆ O
11.	35.782	β -Bisabolol	80867388	0.98	C ₁₅ H ₂₆ O

Table 5: Identified compounds from GC-MS analysis of methanolic extract of *Dennettia tripetala* fruits

Serial No	Retention time (min)	Name of Compound	Peak height	Percentage composition	Molecular formula
1.	5.456	1,2,3,6-tetrahydropyridine	11015176	2.79	C ₅ H ₉ N
2.	7.552	3-Methylpyridine	31352857	4.92	C ₆ H ₈ N
3.	9.910	2(5H)-Furanone	7439289	0.74	C ₄ H ₄ O ₂
4.	11.317	3-Ethylpyridine	22022016	3.08	C ₇ H ₁₀ N
5.	11.724	3-Ethenylpyridine	3914997	0.74	C ₇ H ₇ N
6.	13.025	1,2-Cyclohexanedione	6589607	0.62	C ₆ H ₈ O ₂
7.	14.882	Cis-Aconitic anhydride	21920882	3.07	C ₆ H ₄ O ₅
8.	17.616	3-(α -Hydroxyethyl)aniline	14771109	1.53	C ₈ H ₁₁ NO
9.	17.729	Phenylethylalcohol	20914436	2.69	C ₈ H ₁₀ O
10.	18.573	Benzyl nitrile	20024979	2.41	C ₈ H ₇ N
11.	22.339	N,N'-Dimethyl-2-butene-1,4-diamine	43849156	15.99	C ₆ H ₁₄ N ₂
12.	39.547	1-Heptatriacotanol	34855320	1.61	C ₃₇ H ₇₆ O
13.	39.942	Linoleic acid ethyl ester	97331209	4.78	C ₂₀ H ₃₆ O ₂

Table 6: Identified compounds from GC-MS analysis of methanolic extract of *Ocimum gratissimum* leaves

Serial No	Retention time (min)	Name of compound	Peak height	Percentage composition	Molecular formula
1.	5.425	L-alanine, N-methoxy carbonyl-heptyl ester	3791291	1.18	C ₁₁ H ₂₂ NO ₄
2.	6.469	Furfural	5036704	0.86	C ₅ H ₄ O ₂
3.	6.626	Chloromethylmethyl sulphide	9390788	2.10	C ₂ H ₅ ClS
4.	7.633	2-furanmethanol	11661781	4.20	C ₅ H ₆ O ₂
5.	8.465	4-cyclopentene-1,3-dione	15679074	5.14	C ₅ H ₄ O ₂
6.	9.685	Butyrolactone	2960211	0.92	C ₄ H ₆ O ₂
7.	9.778	2 (5H)-Furanone	13818472	2.81	C ₄ H ₄ O ₂
8.	11.499	5-methyl-2-Furanmethanol,	2561390	0.44	C ₆ H ₈ O ₂
9.	11.611	5-methyl-2-furancarboxaldehyde,	16534458	4.09	C ₆ H ₅ O ₂
10.	11.755	1,1,3,3-tetramethylbutylthiocyanic acid	2072435	0.41	C ₉ H ₁₇ SN
11.	11.893	E-10-dodecyl-1-ol propionate	2049615	0.43	C ₁₅ H ₂₈ O ₂
12.	13.738	Cis-aconitic anhydride	2936559	0.72	C ₆ H ₄ O ₅
13.	14.357	2-hydroxy-3-methyl-cyclopenten-1-one	1523594	0.47	C ₆ H ₈ O ₂
14.	16.484	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	5366495	0.47	C ₆ H ₈ O ₃
15.	17.447	N-Guanylproline	34865203	9.12	C ₆ H ₁₁ O ₂ N ₃
16.	21.782	1-acetyl L-Proline	16655323	4.29	C ₆ H ₁₁ NO ₃
17.	24.222	Thymol	17079291	4.00	C ₁₀ H ₁₄ O
18.	24.754	2-Methoxy-4-vinylphenol	31160365	6.79	C ₉ H ₁₀ O ₂
19.	25.811	2-(1-methyl-2-pyrrolidinyl)-Pyridine	8327015	1.64	C ₁₀ H ₁₅ N ₂
20.	26.249	2-Allyl-4-methylphenol	3307805	0.78	C ₁₀ H ₁₂ O
21.	31.985	[4S-(4R*,5E,10S*)]-3478910-Hexahydro-4-hydroxy-10-methyl-(2H)-Oxecin-2-one	11725773	3.56	C ₁₀ H ₁₆ O ₃
22.	38.778	n-hexadecanoic acid	41567487	4.82	C ₁₆ H ₃₂ O ₂
23.	44.408	6-(1-adamantylamino)-Furazano[3,4-b]pyrazin-5-ol	47193038	11.51	C ₁₅ H ₁₅ N ₅ O ₂

Table 7: Compounds identified common in the three plant extracts

Compounds	% in PG MET	% in OG MET	% in DT MET
2-furanmethanol	2.35	4.20	-
4-cyclopentene-1,3-dione	2.33	5.14	-
2 (5)-furanone	1.85	2.81	0.74
5-methyl-2-furancarboxaldehyde	0.64	0.44	-
Cis-aconitic acid	-	0.72	3.07
n-hexadecanoic acid	10.24	-	4.82

Note: PG MET - *Piper guineense* methanol extract; OG MET - *Ocimum gratissimum* methanol extract; DT MET - *Dennettia tripetala* methanol extract

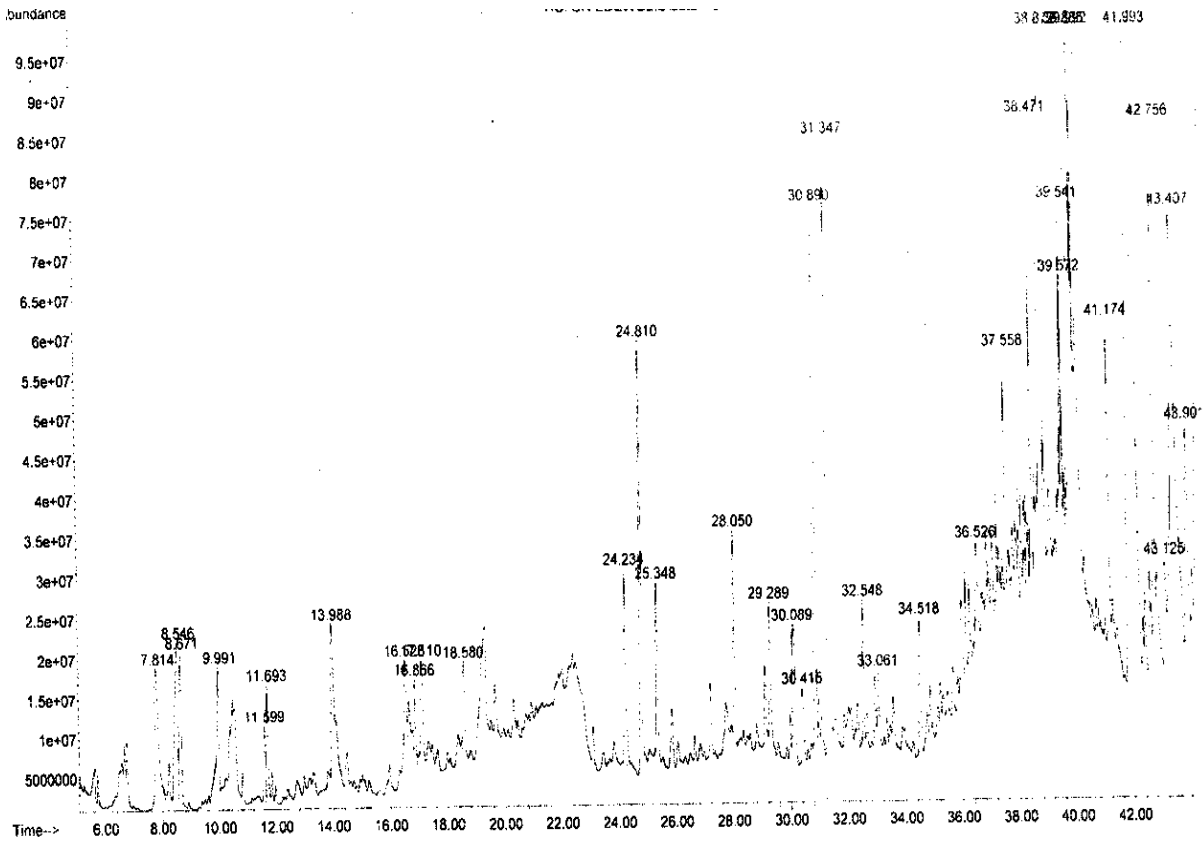


Fig.1a: Total ion chromatogram of methanol extract of *P. guineense* seeds

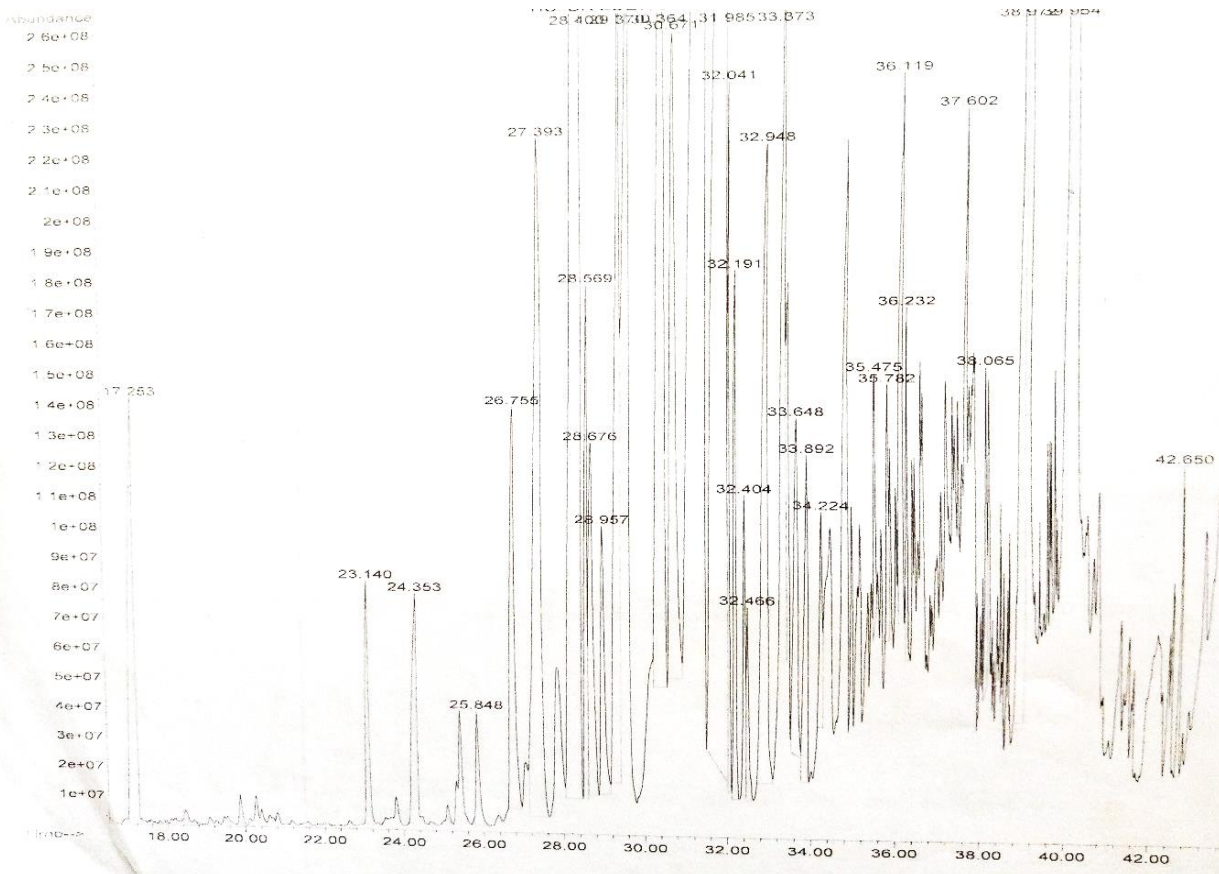


Fig.1b: Total ion chromatogram of hexane extract of *P. guineense* seeds

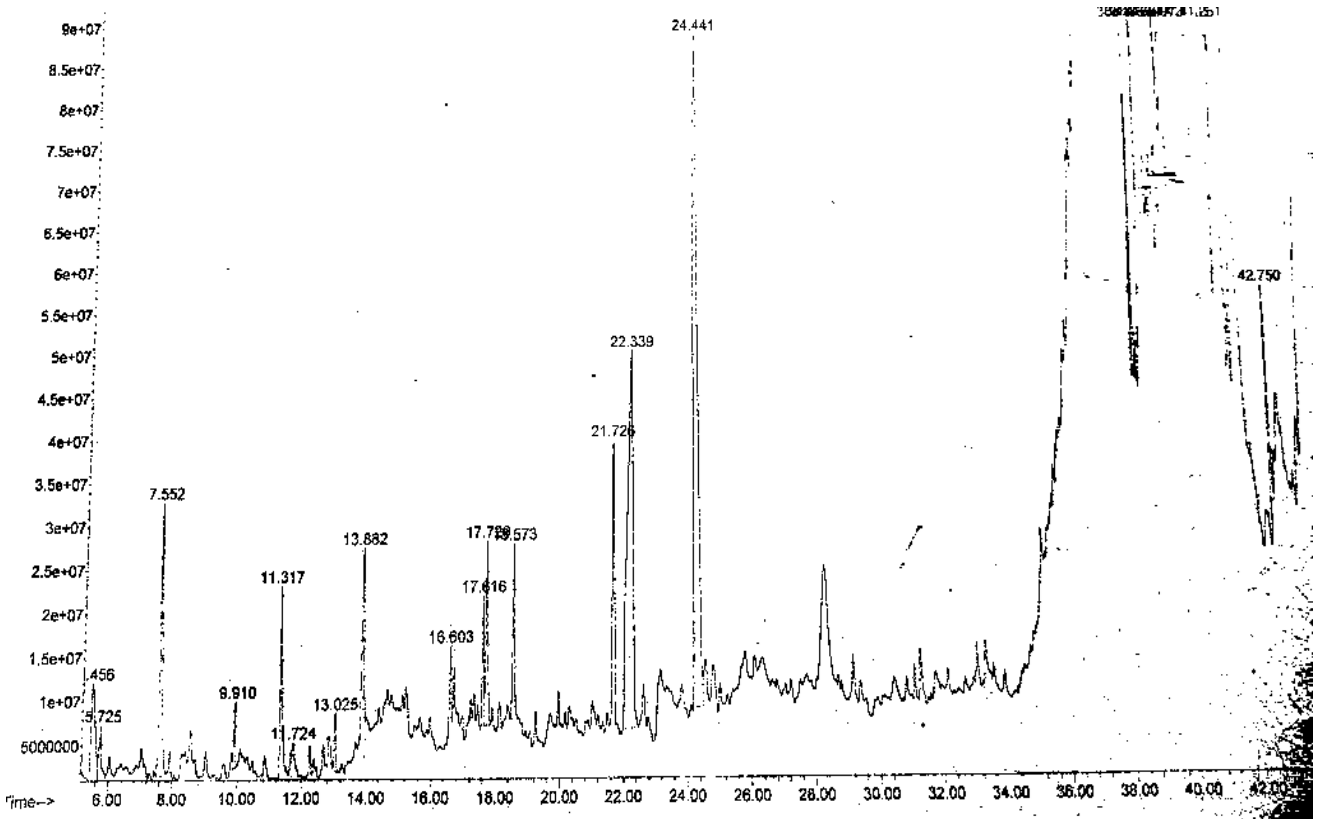


Fig.2: Total ion chromatogram of methanol extract of *D. tripetala* fruits

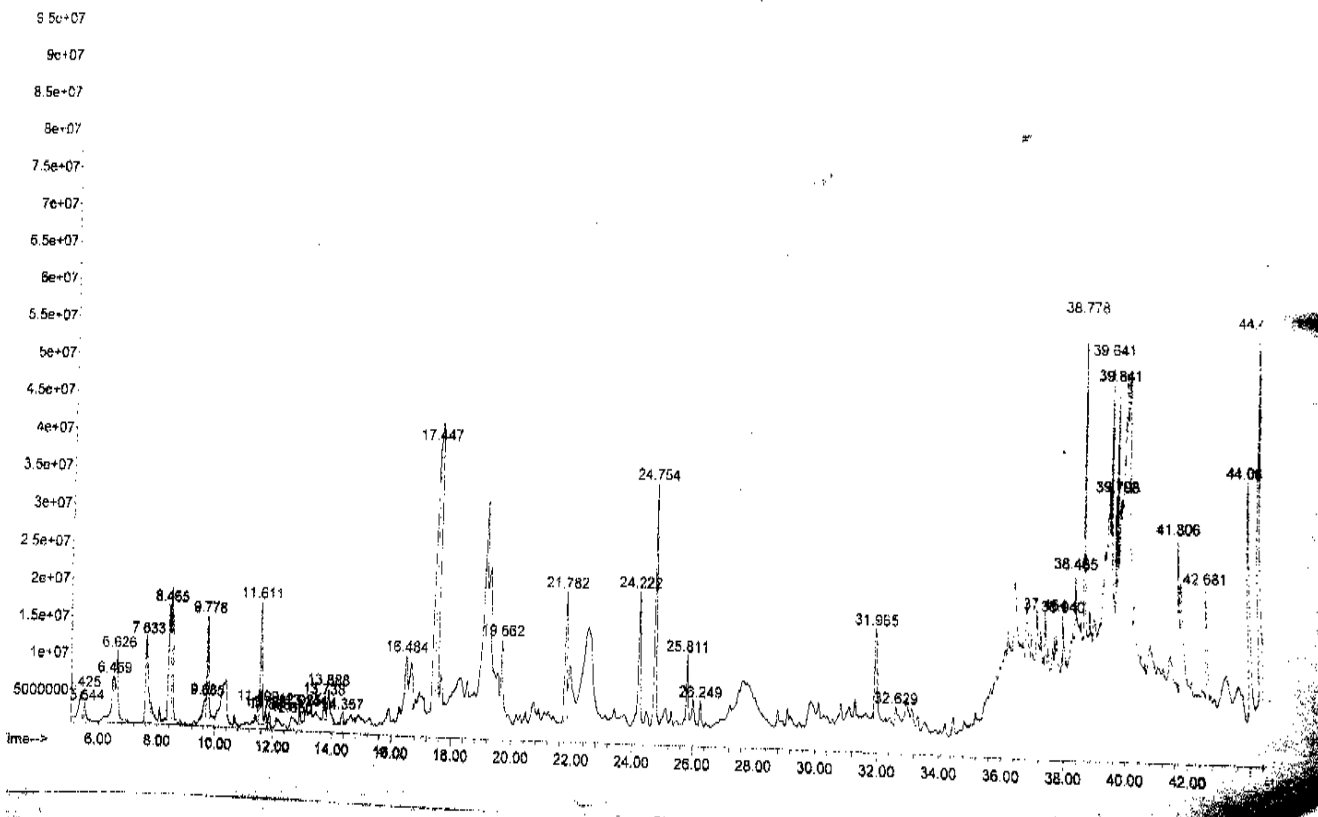


Fig.3: Total ion chromatogram of methanol extract of *O. gratissimum* leaves

IV. DISCUSSION

Pest resurgence, resistance, environmental pollution, lethal effects on non-target organisms are some of the drawbacks associated with the use of synthetic insecticides (Dev and Koul, 1997). The alternative to synthetic is to use natural products and higher plants are a rich source of biopesticides. These biopesticides are considered safer and ecofriendly in that they show selectivity to insect species, are biodegradable and non-toxic to mammalian system. Plant secondary metabolites have been shown to possess some important insect-plant relationships (Isman, 2000, Martinez *et al.*, 2015, Plata-Rueda *et al.*, 2017) and as such can be used to reduce the risk of cross-resistance and serve as new leads for the design of target specific compounds. Therefore, it's imperative to have a good understanding of the type or class of secondary metabolites present in plants or plant parts that possess insecticidal properties.

Phytochemical screening was carried out in order to determine the class or type of chemicals present in the plant samples. The phytochemical analysis of plant extracts from *P. guineense* seeds, *D. tripetala* fruits and *O. gratissimum* leaves as presented in Table 1 showed a strong presence of saponins and tannins in the methanolic extract of *O. gratissimum* leaves which is in consonance with the work of (EL-Mahmood *et al.*, 2008; Elekwa *et al.*, 2011). Flavonoid was also identified present while steroid, alkaloid, glycoside and terpenoid were absent. These are similar to the report of Ameh (2010) on the evaluation of the phytochemical composition and antimicrobial properties of crude methanolic extract of leaves of *O. gratissimum*.

However, variations in the quantities of the phytochemicals present in the extracts were observed in this experiment. Report of n-hexane extract, showed that terpenoid was identified present. For the methanolic extract of *P. guineense* seeds there was strong presence of flavonoid, alkaloid and steroid, and mild presence of terpenoid while tannin, saponin, and glycoside were absent. The n-hexane extract of *P. guineense* seeds showed a moderate presence of steroid and strong presence of terpenoid. The methanolic extract of *D. tripetala* fruits showed presence of flavonoid only, while the n-hexane extract exhibited mild presence of steroid and strong presence of terpenoid. This is in line with reports of Akinwumi and Fesobi, 2010; Elekwa *et al.*, 2011; Ihemeje *et al.*, 2013 on phytochemical constituents, pharmacological and traditional uses of some medicinal plants in the tropics. However, in this experiment terpenoid was observed to be highly present when compared with previous works. According to Onyilagba *et al.*, 2004 flavonoids possess a catecholic B-ring that seems to be responsible for the toxicant activity to insects; therefore the flavonoids present in the methanol extracts of *O. gratissimum* leaves, *P. guineense* seeds and *D. tripetala* fruits could be responsible for their insecticidal property.

The total phenolic content obtained for the extracts were 21.48 ± 0.15 , 67.48 ± 0.12 and 13.09 ± 0.11 mg Gallic acid equivalent / g extract for *O. gratissimum* leaves, *P. guineense* seeds and *D. tripetala* fruits

respectively (Table 2), estimated by using Folin - Ciocalteu reagent and determined from a linear Gallic acid standard curve ($y = 0.023x + 0.292$, $R^2 = 0.946$). This is in line with Amadioha and Chidi (2019) on the determination of flavonoids and phenolic contents of extracts from *P. guineense*, *Cassia alata*, *Tagetes erecta* and *O. gratissimum*. Phenolic compounds have redox properties which allow them to act as antioxidants and this is due to the presence of hydroxyl groups. The extracts have moderate quantity of polyphenols. The total flavonoid content was evaluated by using the aluminum colorimetric assay and quercetin used as the standard. The total flavonoid content was determined from the quercetin standard curve ($y = 0.013x + 0.008$, $R^2 = 0.981$) and the results obtained for the extracts are 0.15 ± 0.01 , 4.23 ± 0.02 and 1.69 ± 0.01 mg quercetin equivalent / g extract for *O. gratissimum* leaves and *P. guineense* seeds and *D. tripetala* fruits respectively. Extracts of *P. guineense* seeds gave the highest level of total phenolic and flavonoid contents while that of *D. tripetala* fruits showed no activity. This was earlier observed by Omodamiro and Ekeleme (2013).

With the GC-MS analysis, MS solution software was used to control the system and acquire data. The identification of the separated compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from National Institute of Science and Technology (NIST) database. The results of gas chromatography-mass spectrometric (GC-MS) analysis of methanolic and n-hexane extracts of *P. guineense* leaves showed that a total of 55 compounds were separated by gas chromatography, out of which 67% of the compounds were identified using the (NIST) National Institute of Standard and Technology database. The total ion chromatograms are shown in figures 1a & 1b. The first compound to emerge from the methanol extract was 2-furanmethanol (2.35%). Compound 31 which was unidentified (retention time: 41.993 min) has the highest percentage in the extract (18.61%). Among the identified compounds n-hexadecanoic acid has the highest quantity in the extract (10.24%). 3 β -stigmast-5-en-3-ol, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-[S-(R*,S*)]-Cyclohexane, β -Bisabolene and 2-methoxy-4-vinylphenol gave moderate quantities (9.27%, 5.1%, 4.0% and 3.84%) respectively. The identified compounds belong to the class of compounds known as steroids, unsaturated hydrocarbons, terpenoids, amino acids, amines and alcohols.

In the gas chromatography-mass spectrometric analysis of methanolic extract of *D. tripetala*, a total of 23 compounds were separated out of which 13 compounds were identified. The total ion chromatogram is given in Figure 2. The first compound to emerge was 1, 2, 3, 6 - tetrahydro pyridine with retention time of 5.46 minutes. Out of the identified compound, N, N'-dimethyl-2-butene-1, 4-diamine gave the highest quantity in the extract (15.99 %). while 1, 2 - cyclohexanedione had the least amount with 0.62%. Among the identified compounds, 3-methyl pyridine and linoleic acid ethyl ester, are those with appreciable quantities in the extract (4.92 and 4.78%)

respectively. The identified compounds belong to the class of compounds known as amino acids, carboxylic acid, fatty acid esters and alcohols. This result is in agreement with those of Rotimi *et al.*, (2018).

In the GC-MS analysis of methanolic extract of *O. gratissimum* a total of 35 compounds were separated while 23 compounds were identified. Its total ion chromatogram is shown in Figure 3.

The first compound to emerge was an amino acid ester, L-alanine, N-methoxycarbonylheptyl ester with retention time of 5.425 minutes while the last to emerge was 6-(1-adamantylamino)-Furazano[3,4-b]pyrazin-5-ol with retention time of 44.408 minutes. This compound has the highest quantity in the extract (11.51%). Other compounds with appreciable quantity in the extract are 2-methoxy-4-vinylphenol (6.79%), 4-cyclopentene-1, 3-dione (5.14%), n-hexadecanoic acid (4.82%), 1-acetyl L-Proline, (4.29%), 2-furanmethanol (4.20%), 5-methyl-2-furancarboxaldehyde (4.09%) and thymol (4.0%). The identified compounds belong to the class of compounds known as carboxylic acids, alcohols, amino acids and fatty acid esters. This is in line with the report of (Al-Marzoqi *et al.*, 2015; Kadhim *et al.*, 2016) which through the use of GC-MS analysis revealed the presence of compounds which were alcohols, carboxylic acids, esters, alkanes and phenols and were suspected to be responsible for the antibacterial activity exhibited by *O. basilicum* and two other herbaceous plants. 2-furan (5H)-furanone was noted common in the methanol extracts of *P. guineense*, *O. gratissimum* and *D. tripetalawhile* 4-cyclopentene-1,3-dione, 2-furanmethanol and 5-methyl-2-furancarboxaldehyde were present in *P. guineense* and *O. gratissimum* with the highest percentage in *O. gratissimum* except in 5-methyl-2-furancarboxaldehyde. Cis-aconic anhydride is present in *O. gratissimum* and *D. tripetala*, and n-hexadecanoic acid in *P. guineense* and *D. tripetala* as presented in Table 7.

The compounds, caryophyllene oxide, β -caryophyllene (Liu *et al.*, 2012) and hexadecanoic acid (Rajashakar and Tonsing, 2014) possess insecticidal properties and these compounds are identified in the methanol extract of *P. guineense* and may be partly responsible for its insecticidal property.

All over the world, there is increase in the rate into which insecticidal botanicals is put in use in order to reduce food poisoning and environmental pollution from the use of synthetic insecticides (Armah, 2011; Bempah *et al.*, 2011). Although there is abundant plant species in the tropics as such farmers can be encouraged into the local preparations of this botanical pesticide. Despite that, it is suggested that further works be carried out on the plant extracts in order to isolate and characterize identified compounds present in the plant extracts so that the isolated compounds can be used for the formulation of insecticides instead of the crude extracts or powder.

V. CONCLUSION

Researches with consistent results from the use of plant materials with beneficial insecticidal properties for the control of crop pests will create the needed platform

and awareness for the adoption and usage of products from such researches all over the world. This will be enhanced when industries and people of means support researches through adequate funding and publicity. Benefits such as reduction in the cases of terminal diseases traceable to synthetic insecticides ingestion through food poisoning is likely to reduce in the society and this will improve the over- all health of man.

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